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Design and development of a multiplex microsatellite panel for the genetic characterisation and diversity assessment of domestic turkey (*Meleagris gallopavo gallopavo*)

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ABSTRACT

Domestic turkey production generally utilises only a few genetically improved lines, and local breeds are severely endangered as a result. Furthermore, the genetic resources of domestic turkeys have not been properly investigated, which could, ultimately, lead to the extinction of local breeds and negatively affect their corresponding genetic diversity and environmental adaptation. Although, several microsatellite markers have been designed for mapping and quantitative trait locus analysis, there is no standard panel of markers for genetic characterisation or genetic diversity assessment. Accordingly, the present study aimed to develop a set of polymorphic microsatellite markers that could be used for international turkey population studies. Thirty-nine microsatellites were selected based on polymorphism, DNA sequence and chromosome position, as well as on amplification efficiency, success rate and the absence of nonspecific amplification. The markers were screened using 105 DNA samples from local turkey breeds from Mexico, the United States, Italy, Brazil, Egypt and Spain. A total of 401 alleles were identified, with a mean number of alleles per marker of 10.28 ± 4.25. All microsatellites were polymorphic, with at least four alleles and no more than 19 alleles. Furthermore, allelic richness ranged from 3.810 to 17.985, mean heterozygosity ranged from 0.452 ± 0.229 to 0.667 ± 0.265, polymorphic information content values ranged from 0.213 (MNT264) to 0.850 (RHT0024) and the mean Fis value was 0.322. Overall, the panel was highly polymorphic and exhibited moderate Hardy–Weinberg disequilibrium, thereby indicating its value as a tool for biodiversity and population structure studies that could play an important role in promoting the conservation of local turkey breeds.

HIGHLIGHTS

- Important genetic resources reside within indigenous turkey populations. These are linked to historic heritage production values and breeds. It is important to preserve this heritage and genetic diversity, which threatens to be lost as production systems focus on production characteristics.
- Microsatellite markers, even though, they are now replaced by single nucleotide polymorphism automatic genotyping platforms in many fields of genetics, remain a viable alternative thanks to their cheapness and simplicity of study which makes them particularly useful when the population to be studied lacks information of the prior genetic structure.

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Supplemental data for this article is available online at here.

Introduction

The domestic turkey (*Meleagris gallopavo)* was domesticated in central America at least 2000 years ago, even if recent research suggests a possible second domestication event in the Southwestern United States between 200 BC and AD 500 (Thornton et al. 2012). All of the main domestic turkey varieties used today are descendants of the turkey raised in central Mexico, which was subsequently imported into Europe during the Spanish colonisation of the southern United States and Mexico during the sixteenth century (Speller et al. 2010). Today, the intensive production of the species is relatively widespread, with numerous genetic lines or varieties and the species is appreciated for its excellent productive performances, which make it one of the greatest sources of meat worldwide. However, many indigenous breeds still exist, even outside the species' original domestication area in Mexico. In fact, following the expansion of the Spanish domains, during the seventeenth century, the turkey was spread throughout Europe and, subsequently, into many countries in the Middle East (Crawford 1992).

The conservation of animal genetic resources is a prerogative of each country, and the morphological, functional and genetic characterisation of breeds is an important first step for this process (Alderson 2018). In turkeys, there are some studies on the phenotypic characterisation at a national level (Adeyemi and Oseni 2017).

Microsatellite markers, despite being progressively replaced by single nucleotide polymorphism markers, remain a valid tool for assessing the genetic diversity of marginal populations in which the number of individuals necessary to describe all the layers of the population is unknown (Vieira et al. 2016) and for which there are no significant genetic resources, as in the bovine species. Microsatellite markers in the turkey genome have been thoroughly studied (Reed et al. 2000; Burt et al. 2003; Chaves et al. 2005), but, the markers have typically been used to develop chromosome and linkage maps for wild species. In addition, only a few biodiversity studies have been conducted, and only few short tandem repeats loci have been identified. Currently, few studies of conservation status and biodiversity of indigenous turkey breeds exist. Colombo et al. (2014) tested the microsatellites recommended by the FAO for chickens and found that 22 of the markers were conserved and informative in the turkey. The aim of the present study was to select and validate a panel of microsatellite markers that could be used for biodiversity studies, paternity assessment and individual identification among local domestic *M. gallopavo*. The present study represents the first attempt to develop a specific panel of molecular markers that can be used for biodiversity studies on turkey.

Material and methods

Sampling and DNA extraction

Blood samples were collected using FTA cards (Flinders Technology Associates, GE Healthcare, Chicago, IL, USA) Samples were collected from 105 unrelated individuals of several local turkey breeds, including Spanish Black turkey ($n = 29$), Spanish Majorquin turkey ($n = 7$), Italian Romagnolo turkey ($n = 24$), Italian Parma turkey ($n = 8$); Mexican Guajolote ($n = 12$), Egyptian turkey ($n = 3$), Brasilian turkey ($n = 2$) and North American turkey ($n = 20$). DNA was extracted from each blood sample by incubating three 2 mm^2 punches of the FTA card in 100 µL of 5% Chelex resin (Bio-Rad, Hercules, CA, USA) suspension at 95 °C for 10 min and then at 99 °C for 3 min. The lysates were centrifuged for 1 min at 2000 rpm, and the resulting supernatants were stored at −20 °C.

Ethics statement

Ethical approval was not needed for this study. Blood samples were collected from local turkey populations by qualified veterinarians during their routine practice within the framework of official programmes aimed at the identification and health monitoring of the breeds and populations included in the present study. The collection did not involve any endangered or protected species. The blood samples were manually collected without injuring the animals, and no other types of tissue (e.g. meat) were used in the present study.

Microsatellite design

A total of 45 loci were selected from existing literature regarding genome mapping and biodiversity assessment in *M. gallopavo* based on (1) distribution in the genome (i.e. among different chromosomes) and (2) sufficient polymorphism in terms of allele richness and heterozygosity, when such information was available. Using the Turkey_2.01 genome assembly (INSDC Assembly GCA_000146605.1, September 2010), the loci were located and 500-bp sequences from before and after the repeated motif were used for primer design.

The primer pairs used for PCR amplification were designed using Primer3 v. 0.4.0 (Rozen and Skaletsky 2000) with the following parameters: optimum length of 20 ± 5 bp, optimum melting temperature of ~60 ± 5 °C, and GC content of 20–80%. The software was also used to check primers from the literature and, eventually, to modify them, in order to achieve melting temperatures of 57–65 °C, to allow more efficient multiplex PCR and to achieve minimum and maximum amplicon sizes of 70 and 450 bp, respectively, which is the optimum range of readability for capillary electrophoresis fragment analysis. Finally, the putative primer sequences were checked for hairpin and self-dimerization using the IDT web tool (https://eu.idtdna.com/calc/analyser) and checked for specificity using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Microsatellite locus amplification

The primers were synthesised by either Integrated DNA Technologies, Inc. (Coralville, IA, USA) or Life Technology (Carlsbad, CA, USA), with HPLC purification and labelling was achieved using the M13-tailed primer method (Boutin-Ganache et al. 2001), in which forward primers are labelled with a specific tail (one for each fluorescent-labelled oligos) and amplified in the presence of four complementary FAM, NED, PET and VIC fluorescent-labelled oligos. Multiplex PCR was performed in three different sets (set one, 14 loci; set two, 15 loci; set three, 10 loci; Supplementary Table 1) with 10 μL reactions volumes, which contained 2 μL of Chelex lysate (~10 ng genomic DNA), 1X MytaqHS 5X buffer (Bioline GmbH, Luckenwalde, Germany), 0.5 U of MytaqHS Polymerase (Bioline), 0.1 μM of each fluorescent M13 oligo, 0.22 μM of each forward M13-labelled primer and 0.2 μM of each reverse primer. The PCR cycle involved the following protocol: 3 min at 95 °C for Taq polymerase activation, 35 cycles of 95 °C for 30 s followed by 3 min at a multiplex-specific annealing temperature, and a final extension step of 60 °C for 20 min. Each annealing step was checked using the gradient function in a Bio-Rad T1000 thermal cycler (Hercules, CA, USA) The lengths of the microsatellite fragments were visualised using an ABI prism 3130 Genetic Analyzer (Life Technology), POP7 polymer and GeneScan500-LIZ (Life Technology) as an internal size standard and the genotypes were determined using ABI Genemapper 5 (Life Technology).

Statistical analysis

Mean allele richness and mean observed and unbiased expected gene diversity, along with their standard deviations and polymorphic information content (PIC), were obtained using MICROSATELLITE TOOLKIT (Park 2001). Nonexclusion probabilities considering the first (NE-1P), second (NE-2P) or parent pairs (NE-PP) and individual (NE-I) and sibling identity (NE-SI) were estimated using Cervus v. 3.0.3 (Kalinowski et al. 2007). Deviations from Hardy–Weinberg equilibrium (HWE) were estimated using Genepop v. 4.0 (Rousset 2008), and *F* statistics were calculated using Genetix v. 4.05 (Belkhir 1999).

Results and discussion

Microsatellite selection and PCR optimisation

Based on amplification efficiency (number of target molecules produced measured as intensity of the fluorescent signal), success rate (absence of amplification failure) and the absence of nonspecific amplification, a total of 39 microsatellite markers were selected for the panel: WT83, TUM20, MNT318, RHT0024, MNT374, WT90-2, MNT331, MNT353, WT75, TUM023, MNT288, MNT264, MNT258, RHT0009, MNT361, MNT266, MNT295, MNT391, MNT360, MNT13, MNT247, MNT411, MNT274, MNT294, MNT348, MNT393, MNT11, MNT014, MNT297, TUM16, WT77-2, MNT386, MNT282, RHT0216, MNT321, WT54, MNT389, MGP-18 and MNT344. Six dinucleotide markers (MNT296, MNT379, MNT409, RHT0131, RHT0174 and MNT412) were excluded due the problems identified above. The general characteristics of the selected loci, along with the corresponding primer sequences and dyes, are presented in Supplementary Table 1. The paucity of information about turkey genetics, especially information about expected allelic range, resulted in unusually large distances between loci. The 39 primer sets were allocated to eight PCR multiplex reactions and to three electrophoresis sets (Supplementary Figure 1). Gradient amplification indicated an optimal hybridisation temperature of 59 ± 0.5 °C, based on the band broadness, for all the multiplex reactions, except the WT75 and MNT13 markers (58 ± 0.6 °C).

Marker polymorphism and quality

A total of 401 alleles were identified and a mean number of alleles for marker of 10.28 ± 4.25. All microsatellites were polymorphic, with at least four alleles (MNT014, MNT288, MNT353 and W77-2) and no more

Table 1. Microsatellite marker panel proposed by the present study.

Locus	Na	Ae	H_O	H_E	PIC	Fis	HW
MGP-18	11	5.188	0.476 ± 0.289	0.811 ± 0.140	0.783	0.414*	**
MNT11	12	2.633	0.519 ± 0.244	0.623 ± 0.242	0.571	0.168*	NS
MNT13	12	4.912	0.571 ± 0.206	0.800 ± 0.134	0.771	0.287*	**
MNT014	4	2.558	0.420 ± 0.150	0.612 ± 0.091	0.542	0.315*	**
MNT247	19	6.784	0.667 ± 0.283	0.857 ± 0.082	0.837	0.223*	**
MNT258	11	2.908	0.437 ± 0.293	0.659 ± 0.225	0.603	0.339*	**
MNT264	5	1.283	0.144 ± 0.167	0.222 ± 0.227	0.213	0.351*	NS
MNT266	16	2.994	0.365 ± 0.228	0.670 ± 0.183	0.617	0.457*	**
MNT274	19	3.040	0.356 ± 0.305	0.674 ± 0.279	0.639	0.473*	**
MNT282	10	2.360	0.452 ± 0.175	0.579 ± 0.187	0.508	0.220*	NS
MNT288	4	2.245	0.437 ± 0.228	0.557 ± 0.235	0.495	0.217*	NS
MNT294	7	3.239	0.552 ± 0.184	0.695 ± 0.191	0.652	0.206*	NS
MNT295	8	4.075	0.598 ± 0.244	0.758 ± 0.270	0.718	0.212*	NS
MNT297	10	2.916	0.419 ± 0.212	0.660 ± 0.221	0.625	0.366*	**
MNT318	5	1.682	0.181 ± 0.178	0.408 ± 0.265	0.382	0.557*	**
MNT321	13	5.109	0.506 ± 0.299	0.809 ± 0.183	0.781	0.376*	**
MNT331	13	6.036	0.640 ± 0.294	0.839 ± 0.137	0.816	0.238*	*
MNT344	14	4.272	0.529 ± 0.291	0.767 ± 0.252	0.733	0.311*	**
MNT348	10	5.283	0.245 ± 0.170	0.815 ± 0.178	0.787	0.700*	**
MNT353	4	1.596	0.305 ± 0.198	0.376 ± 0.222	0.345	0.189*	*
MNT360	7	3.013	0.048 ± 0.056	0.671 ± 0.297	0.608	0.929*	**
MNT361	11	4.179	0.587 ± 0.314	0.764 ± 0.106	0.727	0.234*	*
MNT374	9	2.784	0.408 ± 0.298	0.644 ± 0.248	0.593	0.368*	**
MNT386	12	2.497	0.500 ± 0.336	0.603 ± 0.296	0.583	0.171*	*
MNT389	15	3.212	0.471 ± 0.353	0.692 ± 0.149	0.640	0.321*	**
MNT391	12	2.508	0.439 ± 0.177	0.604 ± 0.095	0.533	0.275*	**
MNT393	16	3.725	0.519 ± 0.280	0.735 ± 0.200	0.703	0.295*	*
MNT411	13	4.701	0.569 ± 0.136	0.791 ± 0.091	0.764	0.282*	**
RHT0009	8	3.778	0.534 ± 0.255	0.739 ± 0.093	0.692	0.278*	*
RHT0024	15	6.482	0.480 ± 0.324	0.850 ± 0.311	0.831	0.436*	**
RHT0216	11	3.192	0.454 ± 0.304	0.690 ± 0.337	0.660	0.344*	*
TUM16	9	2.003	0.346 ± 0.190	0.503 ± 0.207	0.459	0.313*	NS
TUM20	17	5.478	0.630 ± 0.116	0.822 ± 0.105	0.795	0.234*	**
TUM023	6	3.184	0.550 ± 0.126	0.689 ± 0.111	0.637	0.203*	**
W75	6	3.163	0.414 ± 0.190	0.687 ± 0.201	0.634	0.399*	**
W77-2	4	2.768	0.510 ± 0.269	0.642 ± 0.207	0.568	0.207*	NS
WT54	11	3.119	0.563 ± 0.121	0.683 ± 0.094	0.631	0.176*	NS
WT83	4	2.066	0.400 ± 0.225	0.519 ± 0.113	0.460	0.229*	*
WT90-2	8	1.994	0.388 ± 0.216	0.501 ± 0.265	0.461	0.227*	NS
Mean	10.282	3.459	0.452 ± 0.229	0.667 ± 0.192	0.626	0.322	

Locus: marker name; Na: mean number of alleles; Ae: effective number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; PIC: polymorphism information content; Fis: subpopulation fixation Index; HW: Hardy–Weinberg equilibrium; NS: not significative.
*$p < .05$; **$p < .01$.

than 19 alleles (MNT274). The number of effective alleles ranged from 1.283 (for MNT264), to 6.784 (for MNT247), with a mean of 3.459 and the Fis values ranged from 0.168 (for MNT11), to 0.929 (for MNT360). The observed (H_O) and expected (H_E) heterozygosity values were 0.452 ± 0.229 and 0.667 ± 0.265, respectively (Table 1) and PIC values ranged from 0.213 (for MNT264), to 0.850 (for RHT0024). Deviations from HWE were observed in 29 of the 39 loci (Table 1).

Power statistics

The nonexclusion probability values are shown in Table 2. The first two values (NE-1P and NE-2P) give the nonexclusion probability when the parents were considered individually (first and second parent of the opposite sex, respectively). In both cases, the MNT264 locus yielded the greatest values (0.975 and 0.882) and the MNT247 locus yielded the lowest values (0.449 and 0.288). When parent pairs were considered, the results were comparable for identity and sibling identity nonexclusion probability, with the MNT264 locus yielding the highest values (0.975, 0.882 and 0.785, respectively) and the MNT247 locus yielding the lowest values (0.449, 0.288 and 0.120, respectively). The combined exclusion probability (PEC, Table 2) of a marker set indicates the probability of theses markers excluding an erroneous individual, for example a father. In our panel the results showed that in general less than 12 loci are sufficient for any kind of analysis. Combined exclusion probabilities considering the first parent (PEC-1P) showed that only 12 loci are needed to correctly exclude a wrong individual; in the case of the second parent (PEC-2P) six loci are needed and when parent pairs are considerate (PEC-PP), only three are needed. Only two loci would be needed to distinguish an individual in an identity test (PEC-I) while seven are necessary for sibling identity exclusion (PEC-SI).

The aim of the present study was to construct a polymorphic panel of microsatellite markers that could be used in studies of turkey biodiversity. Accordingly, the proposed panel was established using eight local turkey populations, in order to detect as much genetic variation as possible and to produce a panel that would be useful both for genetic diversity and kinship analysis studies. Using published information about microsatellite loci in turkeys, a panel of 45 microsatellite markers was established and 39 of these were selected based on their technical quality. Until the present work, only a few studies had used microsatellite markers in turkeys (Smith et al. 2005; Lopez-Zavala et al. 2013; Seidel et al. 2013). The mean allele richness values observed in the present study were higher than reported by Lopez-Zavala et al. (2013), who investigated seven loci and reported a mean richness value of 9.28. The results of the present study also indicated that the MNT247 and MNT274 loci consistently yielded the greatest number of alleles ($n = 19$), in contrast to the results of Burt et al. (2003), who reported that the markers only yielded seven alleles in Large White turkey. Meanwhile, the allelic richness of the W75 and W77-2 loci in the present study (six and four alleles, respectively) was lower than that reported by Lopez-Zavala et al. (2013), who used the markers for the genetic characterisation of domestic and wild turkey populations in Mexico (Lopez-Zavala et al. 2013). However, when compared to the present study, both studies that were previously cited (Burt et al. 2003; Lopez-Zavala

Table 2. Nonexclusion probability values of the 39 selected microsatellite markers.

Locus	NE-1P	PEC-1P*	NE-2P	PEC-2P*	NE-PP	PEC-PP*	NE-I	PEC-I*	NE-SI	PEC-SI*
MNT247	0.449	**0.551**	0.288	**0.712**	0.120	**0.898**	0.037	**0.969**	0.333	**0.667**
RHT0024	0.459	**0.794**	0.296	**0.915**	0.122	**0.989**	0.039	**0.999**	0.337	**0.888**
MNT331	0.489	**0.899**	0.321	**0.973**	0.144	**0.999**	0.046	1.000	0.344	**0.961**
TUM20	0.524	**0.947**	0.351	**0.990**	0.170	1.000	0.056	1.000	0.355	**0.986**
MNT348	0.538	**0.972**	0.364	**0.997**	0.180	1.000	0.059	1.000	0.359	**0.995**
MNT321	0.547	**0.984**	0.371	**0.999**	0.185	1.000	0.062	1.000	0.363	**0.998**
MGP-18	0.548	**0.991**	0.372	1.000	0.189	1.000	0.062	1.000	0.362	**0.999**
MNT13	0.560	**0.995**	0.384	1.000	0.196	1.000	0.066	1.000	0.368	1.000
MNT411	0.572	**0.997**	0.393	1.000	0.200	1.000	0.069	1.000	0.374	1.000
MNT344	0.612	**0.983**	0.433	0.999	0.238	1.000	0.086	1.000	0.390	0.999
MNT361	0.626	**0.999**	0.447	1.000	0.257	1.000	0.091	1.000	0.392	1.000
MNT295	0.639	**0.999**	0.460	1.000	0.272	1.000	0.097	1.000	0.397	1.000
MNT393	0.650	1.000	0.466	1.000	0.263	1.000	0.100	1.000	0.409	1.000
RHT0009	0.671	1.000	0.495	1.000	0.311	1.000	0.113	1.000	0.411	1.000
RHT0216	0.700	1.000	0.513	1.000	0.304	1.000	0.125	1.000	0.438	1.000
MNT294	0.712	1.000	0.533	1.000	0.339	1.000	0.134	1.000	0.438	1.000
MNT389	0.713	1.000	0.545	1.000	0.354	1.000	0.145	1.000	0.442	1.000
MNT274	0.716	1.000	0.535	1.000	0.328	1.000	0.141	1.000	0.450	1.000
TUM023	0.731	1.000	0.560	1.000	0.378	1.000	0.147	1.000	0.444	1.000
WT75	0.732	1.000	0.561	1.000	0.380	1.000	0.149	1.000	0.445	1.000
WT54	0.735	1.000	0.565	1.000	0.382	1.000	0.152	1.000	0.448	1.000
MNT266	0.736	1.000	0.569	1.000	0.379	1.000	0.161	1.000	0.457	1.000
MNT297	0.736	1.000	0.554	1.000	0.351	1.000	0.150	1.000	0.459	1.000
MNT258	0.750	1.000	0.586	1.000	0.401	1.000	0.171	1.000	0.465	1.000
MNT360	0.755	1.000	0.596	1.000	0.425	1.000	0.171	1.000	0.459	1.000
MNT374	0.760	1.000	0.593	1.000	0.404	1.000	0.177	1.000	0.474	1.000
MNT386	0.773	1.000	0.582	1.000	0.362	1.000	0.177	1.000	0.494	1.000
MNT11	0.783	1.000	0.619	1.000	0.437	1.000	0.194	1.000	0.488	1.000
WT77-2	0.794	1.000	0.647	1.000	0.494	1.000	0.202	1.000	0.481	1.000
MNT391	0.801	1.000	0.659	1.000	0.491	1.000	0.227	1.000	0.506	1.000
MNT014	0.811	1.000	0.664	1.000	0.509	1.000	0.220	1.000	0.500	1.000
MNT282	0.822	1.000	0.683	1.000	0.523	1.000	0.248	1.000	0.524	1.000
MNT288	0.845	1.000	0.700	1.000	0.547	1.000	0.258	1.000	0.537	1.000
TUM16	0.864	1.000	0.714	1.000	0.548	1.000	0.291	1.000	0.572	1.000
WT90-2	0.864	1.000	0.710	1.000	0.540	1.000	0.289	1.000	0.573	1.000
WT83	0.866	1.000	0.726	1.000	0.577	1.000	0.290	1.000	0.564	1.000
MNT318	0.913	1.000	0.770	1.000	0.616	1.000	0.377	1.000	0.641	1.000
MNT353	0.928	1.000	0.800	1.000	0.666	1.000	0.421	1.000	0.668	1.000
MNT264	0.975	1.000	0.882	1.000	0.785	1.000	0.615	1.000	0.793	1.000

NE-1P: nonexclusion of one candidate parent; NE-2P: candidate parent given the genotype of a known parent of the opposite sex; NE-PP: candidate parent pair; NE-I: identity of two unrelated individuals; NE-SI: identity of two siblings; PEC: combined exclusion probability calculated using the Jamieson formula (Jamieson 1994).
Markers needed to reach the target combined probability of 0.999 are in bold and underlined.

et al. 2013), were limited in the number of markers and in sample size. In the present study, the analysis of 39 markers revealed a high mean Fis value (0.322). Observed heterozygosity (mean 0.452 ± 0.229) ranged from 0.048 (MNT360) to 0.667 (MNT 47), which indicated that the marker panel exhibited relatively low polymorphism (Ott 1992). Indeed, previous microsatellite studies have reported mean H_O values of 0.533 in 144 samples (Lopez-Zavala et al. 2013), whereas Smith et al. (2005) reported a mean H_O of 0.73 in 94 samples of five varieties of domestic turkeys from the United States. Because studies of turkey genetics are relatively limited, it might also be useful to compare the results of the present study with those of studies focussed on chickens. For example, Ceccobelli et al. (2015), who studied 16 European chicken breeds, and Granevitze et al. (2007), who studied 64 chicken populations, estimated H_O values of 0.456 and 0.460, respectively, which are very close to the findings of the present study. The expected frequencies of heterozygotes per locus (Table 1) ranged from 0.222 (MNT 264) to 0.857 (MNT 247), and the mean H_E values were generally moderate (0.667 ± 0.1912) but higher than those previously reported in turkey. For example, Lopez-Zavala et al. (2013) reported a mean H_E value of 0.560, which is similar to the H_E value reported by Granevitze et al. (2007) for chickens (0.520) and lower than that reported to native chickens in Korea (Kong et al. 2006). The Hardy–Weinberg disequilibrium results may indicate the presence of population stratification within a sampled population that can lead to equilibrium deviation. Other factors that contribute to Hardy–Weinberg disequilibrium include selection and inbreeding, in the case of closed populations. (Granevitze et al. 2007; Blackburn et al. 2011; Montenegro et al. 2015).

In most cases, acceptable combined probabilities for marker sets, in an exclusion process using codominant markers, should be between 0.997 and 0.9999

(Okada and Tamate 2000; Liu and Yao 2013). Hence, considering the first and second parent of the opposite sex (given the genotype of the first parent) with the first 12 and nine respectively most informative loci we can exclude the wrong parent (Table 2). The combined exclusion probability for a candidate parent pair that exhibits an acceptable probability of exclusion, is near 100%, with the four most informative loci, whereas combined exclusion probabilities for the identity of two unrelated individuals and the combined exclusion probability for the identity of two siblings requires three and six loci, respectively. According to these results, a panel of $\geq$12 of the most informative markers is sufficient for correct parentage and identification analysis in *M. gallopavo*, as previously reported for other farm animal species, like cattle (Schnabel et al. 2000; Stevanovic et al. 2010), chickens (Olowofeso et al. 2016) and horses (Cho and Cho 2004).

Conclusions

In the present study, a large panel of microsatellite markers was developed and confirmed for use in turkey diversity studies. The panel consists of 39 polymorphic loci, which were selected for optimal distribution in the *M. gallopavo* genome and for efficient reproducibility in the laboratory. Statistical analysis indicated a high degree of polymorphism and moderate degree of Hardy–Weinberg disequilibrium, which indicated that the panel was suitable for biodiversity and population structure studies. For the first time, a standardised tool is available for the international meta-analysis of the turkey's genetic substructure at the international level. Further, the PEC results showed that a relatively low number of markers are needed for parentage and identification studies

Ethical approval

Blood samples from all animals incluted in this study were collected by qualified veterinarians during their routine practice within the framework of official programs aimed at the identification, monitoring of health, and parentage confirmation of the breeds and populations included in our study. The collection. The fieldwork did not involve any endangered or protected species. Hair root were manually collected without any injury in the back of the animals No other kind of tissues (blood, meat or other) were used in this study. The other breeds are data proceded from other study inside our research group.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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